monocytes. While many studies have shown that MSCs impair the differentiation of monocytes into dendritic cells and macrophages, there are few articles showing the interaction between MSCs and monocytes and none of them have addressed the question of monocyte subsets modulation.
Methods: To better understand the mechanism behind the benefit of MSCs infusion for graft-*versus*-host treatment through monocytes involvement, we performed mixed leucocytes reactions (MLR) in the presence or absence of MSCs. After three or seven days, cultures were analyzed by flow cytometry using different approaches.
Results: MSCs induced changes in monocyte phenotype in a MLR. This alteration was accompanied by an increase in monocyte counting and in CD14 expression. MSCs induced monocyte alterations even without contact, although the parameters above were more pronounced with cell-cell contact. Moreover, the presence of MSCs impaired MHC I and II, CD11c, CCR5 expression and induced CD14 and CD64 expression on monocytes. These alterations were accompanied by a decrease in IL-1β and IL-6 production by these monocytes but no change was observed taking into account the phagocytosis capacity of these monocytes.
Conclusions: Our results suggest that MSCs impair the differentiation of $CD14^{++}CD16^{-}CD64^{+}$ classical monocytes into $CD14^{++}CD16^{+}CD64^{++}$ activate monocytes, having a role even earlier than the differentiation of monocytes into dendritic cells and macrophages.
Keywords: Immunosuppression, Mesenchymal stromal cells, Monocyte subsets
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169

EFFECTIVE MOBILIZATION OF MESENCHYMAL STEM CELLS IN C57BL/6 MICE UTILIZING SINGLE AGENT PLERIXAFOR (AMD3100) OR IN COMBINATION WITH NEUPOGEN (G-CSF)

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Introduction: Mesenchymal stem cells (MSC) are a rare population of cells that have the ability to form muscle, bone, cartilage, and adipose. MSC can be obtained from the bone marrow (BM). Strategies to mobilize MSC into the peripheral blood (PB) where they can be easily collected would be of therapeutic benefit but there is a lack of consensus on effective strategies to mobilize MSC.
Methods: During a screen in C57BL/6 mice, subcutaneous (SC) injections of neupogen (G-CSF) or plerixafor (AMD3100) were identified as agents that mobilize MSC. Subsequently, neupogen (G-CSF, 50μg/kg SC twice a day for 4 days) and plerixafor (AMD3100, 5mg/kg SC, once 1 hr prior) were assessed in individual mice (N = 5) as single agents or in combination. PB and BM were collected and plated for colony formation in 5%CO_2 5%O_2 for 7 days. MSC colonies were scored as colony formation units-fibroblast (CFU-F total, large, & small) in parallel with hematopoietic progenitors (CFU-GM, BFU-E, and CFU-GEMM). Data is presented as colonies /mL PB or /femur ± SEM and analyzed using Mann-Whitney *U* test.
Results: Analysis of data showed that, as compared to saline, treatment with G-CSF and to a greater extent AMD3100 resulted in mobilization of total CFU-F (large & small CFU-F) into the PB (5.5±2.5, 22.1±5.3, and 135.8±11.5 CFU-F/mL respectively ($p<0.05$). Combination G-CSF+AMD3100 mobilized at levels (110.5±5.1 CFU-F/mL) not statistically different than single agent AMD3100 ($p<0.05$). Analysis of the large CFU-F colonies revealed that a significant number of large CFU-F were mobilized with AMD3100 or combination G-CSF+AMD3100 ($p<0.05$), but not G-CSF alone. In the BM, the total number of CFU-F, as compared to saline, was decreased in response to G-CSF or G-CSF + AMD3100, but not single agent AMD3100 ($p<0.05$). The response of hematopoietic progenitor mobilization to the agents tested was as expected: G-CSF, AMD3100, or G-CSF+AMD3100 mobilize CFU-GM, BFU-E, and CFU-GEMM into the PB with the greatest level of mobilization resulting from G-CSF+AMD3100 ($p<0.05$).
Conclusion: Single agent AMD3100 administered SC is effective at mobilizing mouse MSC (total CFU-F or large CFU-F) with no additional enhancement from combination G-CSF+AMD3100. G-CSF treatment, alone or in combination, depletes MSC in the bone marrow whereas treatment with AMD3100 does not. This suggests that use of single agent plerixafor (AMD3100) may be an effective strategy to mobilize MSC for use in the context of regenerative medicine.

170

EPAC ACTIVATION REGULATES HUMAN MESENCHYMAL STEM CELLS MIGRATION AND ADHESION

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Currently, one of the challenges confronted by the clinical applications of human mesenchymal stem cells (hMSCs) is how to enhance the homing and engraftment of hMSCs to the target tissues with high efficiency. To overcome such barrier, mechanisms responsible for the hMSCs homing and engraftment are one of the key research foci. As of now, the exact mechanism and soluble factors involved in migration and adhesion of hMSCs have not been completely unfolded. Exchange protein directly activated by cAMP (Epac), a novel protein discovered in cAMP signaling pathway, attracts our attention due to its potential role in regulating cells adhesion and migration by triggering the downstream Rap family signaling cascades. However, the exact biological role of Epac in cells homing remains elusive and even controversial. Our study aimed to evaluate the regulatory effects of Epac in the homing process of hMSCs. We confirmed that hMSCs expressed functional Epac. In addition, Epac activation stimulated by specific analogue enhanced the adhesion and migration capacities of hMSCs significantly. Such homing enhancement effects were associated with corresponding morphological changes induced by Epac. The Epac activation was further found to be contributed directly to the chemotactic responses induced by stromal cell derived factor-1 (SDF-1) which is a known crucial chemokine in regulating hMSCs homing. These findings suggested Epac is connected to the SDF-1 signaling cascades. In conclusion, our study revealed that Epac plays a role in hMSCs homing by promoting adhesion, migration and also by enhancing chemotactic effect induced by SDF-1. Appropriate manipulation of Epac may enhance the homing and engraftment of hMSCs and facilitate the future clinical applications of hMSCs.

171

ROLE OF NFATC2 IN PROLIFERATION AND DIFFERENTIATION OF HUMAN CD34+ HEMATOPOIETIC STEM CELLS

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Background: Cyclosporine-A (CSA) inhibits NFATc2 activation and is effective in management of graft versus host disease (GVHD). Little is known of the role of NFATc2 transcription factor and its inhibition, in affecting differentiation and proliferation of CD34+ HSC's (Hematopoietic Stem Cells).
Methods: We used Ficoll density gradient method to obtain Mononuclear Cell (MNC) from human cord blood units (provided by Pablo Rubinstein, MD, New York Blood Center). MNC's were enriched for CD34+ HSC's using magnetic bead separation (Auto Macs, Miltenyi) which were plated at density of 60,000 cells / ml in serum free media with IL-3 (5ng/ml), FLT-3L (100 ng/ml), SCF (50 ng/ml), G-CSF (30 ng/ml) and GM-CSF (5ng/ml). Cells were cultured under normoxic conditions for 7 days at 37.0^{C} with 5% CO2. HSC proliferation and differentiation was studied in presence and absence of CSA.
Results: Flow cytometry analysis of HSC's grown in absence of CSA showed high CD 34 (84.7%) and low CD33/ HLA-DR (0.57% and 5.71 % respectively) expression on Day 0 of culture. In presence of CSA at 2mM concentration, we noted a more rapid rise of CD33 (41.0% versus 28.1%) HLA-DR (44.4% versus 29.1%) and CD 71 (12 % Vs 24.4%) markers by day 7. No difference was noted in total cell count in CSA treated 701,666.6 ± 7637 (mean and SD) and untreated 643,333 ± 5166 conditions.